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Endogenous Short Period Rhythms in the Movements of  
Unifoliate Leaves of *Phaseolus angularis* Wight.<sup>1,2</sup>

<sup>3</sup>  
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Abstract. Rhythmic rotational movements with the midvein as the  
axis have been observed in the unifoliate leaves of *Phaseolus*  
*angularis* Wight grown under controlled environmental conditions

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1 with continuous light. The mean period of this movement for all  
2 leaves was 53.2 min.  $\pm$  4.3 min. and remained constant as the leaf  
3 matured, except after removal of the apical meristem and emerging  
4 trifoliate leaf when the period increased about 5 min. The ampli-  
5 tude of the movement also remained constant as the leaf matured.  
6 These rotational movements were pronounced when the leaf blade  
7 was in a horizontal position and were not evident during the down-  
8 ward or "sleep" movements of the leaf. This movement began 3 days  
9 after leaf unfolding and continued for at least 6 days. It was  
10 most pronounced at the time of inflection of the leaf length growth  
11 curve after the logarithmic phase of growth.

12  
13 The unifoliate leaves of bean plants are generally in constant motion  
14 and they display distinct patterns as they move. This study describes an  
15 oscillatory type of leaf movement in which the lamina rotates back and  
16 forth about the midvein axis, with the direction of rotation changing  
17 every few minutes. This type of motion has been observed only briefly by  
18 other investigators and has not been accurately characterized. Darwin  
19 and Darwin (6) noted rotational movements of bean leaves but only when the  
20 leaves were downward or in a "sleep" position. They designated this type  
21 of motion as "blade rotation." Tronchet et al. (14) reported blade rotation  
22 in the unifoliate leaves of *Phaseolus* with periods of about 1 hr., but  
23 these observations were limited to only a few movement cycles.

24 In contrast to rotational movements, upward, downward and circumnu-  
25 tational movements of leaves have been reported frequently in the literature.

1 The upward and downward movements commonly have a dominate period of about  
2 27 hrs. and less prominent irregular motions of small amplitude with  
3 periods of about 1 hr. (5). The 27 hr. movements have been observed in  
4 bean leaves for as long as 4 weeks (9). These movements originate spon-  
5 taneously in young seedlings grown under controlled environmental condi-  
6 tions of temperature, light, CO<sub>2</sub> and relative humidity (1). Circumnuta-  
7 tional movements have been observed most commonly in specialized leaves  
8 called tendrils (7,11,12) in which the tendril tips follow a more or less  
9 regular ellipse as they move. Darwin and Darwin (6) also noted movements  
10 of this type in the true leaves of many plant species. The leaf tips of  
11 *Phaseolus vulgaris* circumnutate approximately once each hr (14).

12 This investigation describes in detail the nature and particularly  
13 the rhythmicity of blade rotation during the growth and maturation of the  
14 unifoliate leaves of *Phaseolus angularis*.

#### 16 MATERIALS AND METHODS

17  
18 The leaf movements were recorded by time lapse photography. *Phaseolus*  
19 *angularis* was considered desirable for this type of study because of its  
20 small, ovate unifoliate leaves and slow growth characteristics. The  
21 plants were grown under continuous light of  $600 \pm 15$  ft-c, a temperature of  
22  $24.0 \pm 0.5$  C, a relative humidity of  $84 \pm 4\%$  and sealed in a plexiglass  
23 chamber to provide controlled conditions of CO<sub>2</sub>, water availability and  
24 mineral nutrition. Carbon dioxide exhaustion was avoided by metering a  
25 constant flow of air from a compressed air cylinder continuously into the

1 chamber during the growth of the plants. The plexiglass chamber was main-  
2 tained in a room of the Biotron, a controlled environment facility of the Uni-  
3 versity of Wisconsin. Entry into the plexiglass chamber was required only  
4 at the start of photographic recording for orientation of seedlings and once  
5 8 days after leaf unfolding to remove the apical meristem and trifoliate  
6 leaves. The details involved in environmental control and cultural practices  
7 were described in a previous publication (1). Plant response data were taken  
8 from leaves of 4 plants in two experiments and from the leaves of 3 plants  
9 in a third experiment. A 16 mm camera provided time lapse photographs  
10 of the leaf positions at 6 min. intervals. Photographic recordings were  
11 started at the time of leaf unfolding and all plants were monitored for  
12 a period of 2 weeks.

13       Measurements of blade rotation were obtained from time lapse photo-  
14 graphs of each bean plant which recorded lateral views of the unifoliate  
15 leaves as they moved. As the leaves rotated, varying portions of the upper  
16 or lower surface were visible to the camera. By measuring the distance  
17 in mm. between the apparent lateral margins of each leaf of the  
18 projected image, it was possible to obtain an accurate relative determin-  
19 ation of the rotation at any time. If the leaf rotated so that the upper  
20 surface of the leaf was exposed, positive values were recorded; if the  
21 leaf rotated in the opposite direction exposing the lower surface, negative  
22 values were recorded. It should be noted that these blade rotation  
23 measurements are relative and will increase with a given intensity of  
24 rotation as the blade expands in width. Movement data also were obtained for  
25 upward and downward circadian movements of the same leaves so that compari-  
sons between the 2 types of movement could be made.

## RESULTS AND DISCUSSION

A rhythmic rotational type of movement with the midvein as the axis was observed in the primary leaves of *Phaseolus angularis* Wight. The term "blade rotation", as previously designated by Darwin and Darwin will be used (6). The rhythmic nature of blade rotation can be seen in Fig. 1. Blade rotation was persistent and self-sustaining under uniform environmental conditions and thus fits the definition of a true biological rhythm (13).

In contrast to the observations of Darwin and Darwin (6), blade rotation occurred only when the leaves were in an upward position and tended to fade out when the leaves were downward or in a "sleep" position (fig. 1). As a result, when blade rotation data was plotted versus time, it was apparent that periods of rhythmic activity were separated by periods of inactivity. Because of these interruptions in activity, only daily periods having 4 or more rhythmic cycles were included in the determination of the average period and amplitude of blade rotation. Whenever the leaf blade exhibited no measurable rotation for 12 or more minutes it was considered to be inactive. An average of 10 and a maximum of 19 cycles per daily period of activity were observed. The average period of blade rotation was calculated for each of these successive daily periods of rhythmic activity.

The overall mean period of blade rotation for the 11 plants, representing a total of 587 cycles, was 53.2 min. There was no significant variation between plants or experiments. The mean period of movement for individual plants varied no more than  $\pm 4.3$  min. from the 53.2 min. mean

1 for all plants (Table I). Analysis of variance indicated that during the  
2 early stages of plant growth, the period of blade rotation did not change  
3 significantly. However, there was a 4.7 min. increase in period immediately  
4 following removal of the apical meristems and trifoliate leaves (Table II).

5 Blade rotation activity was not observed until 3 days after leaf unfolding,  
6 when activity began and persisted for at least 6 days. The movement decreased  
7 considerably and eventually disappeared as the plant aged. This is evident  
8 in Fig. 2, in which the average relative rhythmic activity of 11 plants is  
9 plotted together with data on elongation of leaves. Relative rhythmic activity  
10 was obtained by dividing the number of rhythmic cycles for each daily period  
11 by the number of rhythmic cycles in the most active daily period for each plant.  
12 Rhythmic activity was generally observed for at least 6 days and for as long  
13 as 9 days in some plants. This is in contrast to circadian rhythm activity  
14 which began immediately as the leaves unfolded and was observed for as long  
15 as 28 days (9).

16 There appeared to be a close relationship between stages of leaf development  
17 and blade rotation activity. Rhythmic activity was most intense during the  
18 inflection in growth rate of leaves just following the logarithmic phase of  
19 growth (fig. 2). Activity decreased and the movement became non-rhythmic as the  
20 leaf attained its maximum size. As the rhythmic activity decreased in the  
21 unifoliate leaves, rapid growth and expansion were occurring in the young  
22 trifoliate leaves.

23 A reasonably precise calculation of degrees of rotation was determined  
24 from the collected data. The degrees of rotation were obtained as a sine value  
25 by dividing the measured mm of blade rotation by leaf width. Leaf width could

1 not be measured directly but was determined from a plotted curve of leaf  
2 length (measured on the photographs) versus leaf width developed for this  
3 cultivar. It was determined that deviations from the horizontal position  
4 averaged  $20^{\circ}$  for each rhythmic cycle with many as great as  $40^{\circ}$ . The average  
5 amplitude of blade rotation did not change from day to day. During later  
6 stages of development, the movement continued with the same amplitude but  
7 became non-rhythmic.

8       It was observed that blade rotation movements for the 2 leaves on the  
9 same plant were closely synchronized and in phase (fig. 3). This is in  
10 contrast to circadian movements for leaves on the same plant, which can be  
11 several hours out of phase (9). The synchronization of blade rotation is  
12 noteworthy since Baillaud (3) stated that closely synchronized short period  
13 movements of leaves on the same plants have not been observed. On the other  
14 hand, the results of this study demonstrate that the rotational movements of  
15 leaves of separate plants were not synchronized (fig. 1), indicating that this  
16 short period rhythm was not entrained to some concurrent environmental fluctuation.

17       It was determined, by watching the orientation of marks made on the upper-  
18 side of the petiole, that most of the blade rotation motions can be attributed  
19 to activity in the secondary pulvinus and to a lesser extent in the primary  
20 pulvinus. In some time lapse records plants have been orientated so that the  
21 tip of the leaf was pointed toward the camera. It was evident that during blade  
22 rotation the leaf blade moved laterally back and forth so that the leaf tips  
23 formed arcs or hemispheres as they moved. However, the leaf tips did not circum-  
24 scribe elliptical or ovoid motions typical of circumnutation.

25

1 It would be interesting to speculate on the nature of the controlling  
2 mechanisms of blade rotation. It is suggested that because of the rapidity  
3 of blade rotation, the movements of the pulvinus must be associated with  
4 changes in turgor on its opposite sides. Since auxin has been implicated in  
5 the turgor movements of pulvini, it may be that a rhythmic fluctuation in auxin  
6 concentration on opposite sides of the midvein is the controlling factor in  
7 blade rotation.

8        Stomatal apertures may regulate water levels within the pulvinus and  
9 thus exert some control on blade rotation. Stomatal rhythms have recently  
10 been observed in Phaseolus with periods of between 40 and 50 min (8),  
11 which closely approximate the timing of blade rotation. It is possible that  
12 the 2 rhythms are closely synchronized and perhaps stomatal rhythms strongly  
13 modify or control these rhythmic leaf movements.

## ACKNOWLEDGMENTS

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10 spontanés des premières feuilles épicotylées de plantules de Phaseolus  
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1 Fig. 1. Movements of primary leaves for 2 separate *Phaseolus angularis*  
2 plants grown simultaneously. (a) blade rotation in millimeters  
3 as recorded by measuring the distance between the lateral margins  
4 of the leaf as it appeared on the projected image (b) upward  
5 and downward movement in angular degrees.

6 Fig. 2. (a) Mean length of unifoliate leaves of *Phaseolus angularis*  
7 plants for 12 days after leaf unfolding (b) rhythmic activity of  
8 blade rotation for 12 days after leaf unfolding. Rhythmic activ-  
9 ity was obtained by dividing the number of rhythmic cycles for  
10 each daily period by the number of rhythmic cycles in the most  
11 active daily period.

12 Fig. 3. Synchrony in blade rotation of the 2 unifoliate leaves on a bean  
13 plant.  
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Table I. Mean Period for Blade Rotation (min) of  
Leaves of *Phaseolus angularis* Plants Grown  
in 3 Separate Experiments

Plant	Experiment		
	A	B	C
1	54.0	53.1	54.8
2	57.5	50.7	55.1
3	52.7	52.5	54.0
4	51.4	49.5	--

<u>F Values</u>	
Plants	1.46 ns
Experiments	2.20 ns

Table II. Mean Period of Blade Rotation (min) for  
Successive Daily Periods of Rhythmic Activity

Period of Blade Rotation (Min)
52.0 (a)
49.8 (a)
52.0 (a)
53.8 (a)
⇒ 58.5 (b)

Figures followed by the same letter are not significantly different  
at the .01 level. (Duncan's Multiple Range Test)

⇒ indicates time of removal of apical meristem and trifoliate leaves.

Figure 1.

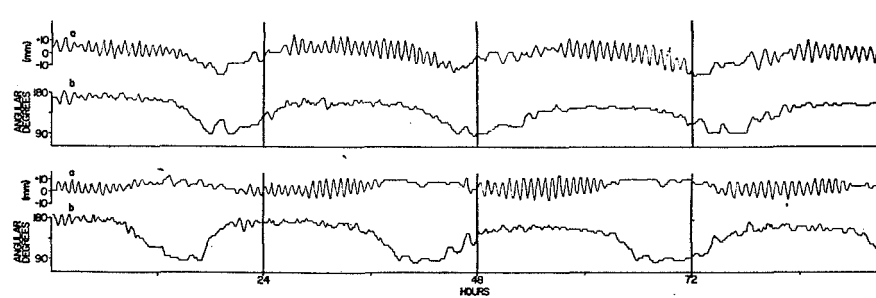


Figure 2.

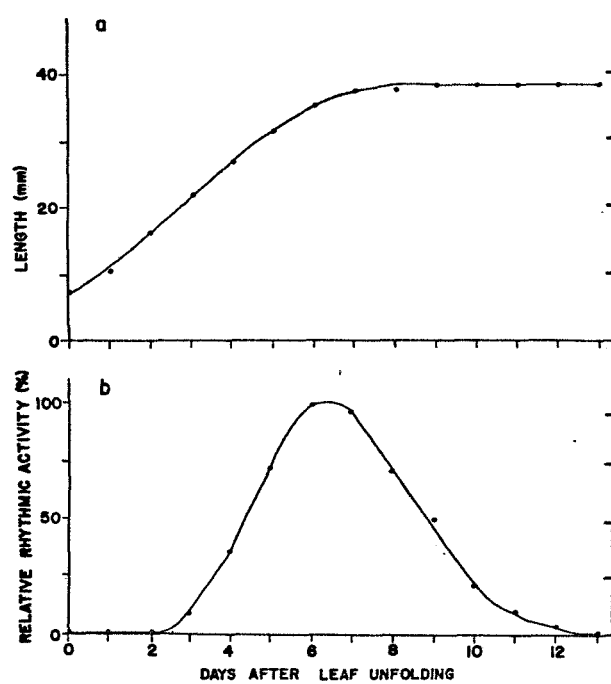


Figure 3.

